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The biogenic production and subsequent oxidation of methane in aquatic sediments are important microbial processes that contribute to the global carbon cycle and play a significant role in the natural flux of methane to the atmosphere. I studied the importance of biogenic methane to the diet of benthic *Chironomus* as well as the effects of sedimentation on *Chironomus* carbon signatures through the use of stable isotope analysis. Results suggest benthic *Chironomus* are obtaining a portion of their carbon (4 – 89%) from biogenically produced methane via methane-oxidizing bacteria (MOB). Core incubation experiments showed significant levels of methane production (up to 1,514.7 $mg/m^2/day$) and methane oxidation (up to 1101.0 $mg/m^2/day$). Sedimentation rates, measured with vertical-suspended sediment traps, were variable by site, reservoir and thermal profile. Linear regression analysis found a significant negative correlation between total sedimentation and the percent organic matter in the sediment and a positive relationship between inorganic sedimentation and the $\delta^{13}C$ values of *Chironomus*. In conclusion, biogenic methane and sedimentation are important to *Chironomus* in these reservoirs, and the production and oxidation of biogenic methane in these reservoirs is significant.

THE IMPORTANCE OF BIOGENIC METHANE AND SEDIMENTATION TO BENTHIC CHIRONOMID LARVAE IN FOUR RESERVOIRS

by

Elizabeth M. Wade

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Approved by

Anne E. Hershey Ph. D.
Committee Chair

To my parents, Art and Cathy Mendoza for their never-ending support and enthusiasm throughout my academic career, and to my husband, Bradford for all his support during the many long days and nights of work pursuing my graduate degree.

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair ____Anne. E. Hershey_Ph. D._____

Committee Members ____Parke Rublee Ph. D._____

____John O'Brien Ph. D._____

____Steve Whalen Ph. D._____

_____
Date of Acceptance by Committee

_____
Date of Final Oral Examination

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CHAPTER I

INTRODUCTION

Methane (CH_4) is a powerful greenhouse gas, currently accounting for nearly one third of the greenhouse effect experienced today, and is approximately 25 times more efficient at trapping heat in the atmosphere on a per molecule basis than carbon dioxide (CO_2) (IPCC 2001). Biogenic methane, produced by microorganisms, is emitted from habitats such as aquatic sediments in reservoirs and lakes, landfills, livestock feedlots, rice paddy fields and wetlands (Bunn & Boon 1993, Huttunen et al. 2002, Fearnside 1997, St. Louis et al. 2000, Duchemin et al. 1995, Kajan & Frenzel 1999, Lassey et al. 1997). It can become incorporated into living tissues via microorganisms that consume methane (Hershey et al. 2006, Oremland & Culbertson 1992, Whiticar & Faber 1986), or be released into the atmosphere through diffusion or ebullition (St. Louis et al. 2000, Keller et al. 1994). Recent research suggests that reservoirs constitute a substantial contribution to greenhouse gas emissions through the emission of methane gas (Fearn et al. 1997, Duchemin et al. 1995, Keller & Stallard 1994, Huttunen et al. 2002, Venkiteswaran & Schiff 2005, Kelly et al. 1997). In a recent review, St. Louis et al. (2000) noted that the global average areal methane flux from reservoirs to the atmosphere was approximately 120 $mg/m^2/day$, whereas natural lakes averaged only 9 $mg/m^2/day$, indicating that reservoirs are significant anthropogenic sources of greenhouse gas emissions.

Reservoirs are unique ecosystems with characteristics of both lakes and rivers. They are typically dendritic or elongated as compared to the more rounded or oval shape of most natural lakes. Reservoirs in warmer temperate areas are usually polymictic or are monomictic if they are deep enough to stably stratify, whereas natural lakes are primarily dimictic in temperate zones (Kalff 2002). With less vegetation in the catchments and more shoreline development, reservoirs usually have higher amounts of inorganic particulate matter than natural lakes (Wetzel 2001). The "fingers" or elongated basins of a reservoir are more riverine, with high terrestrial inputs of nutrients and high sediment load. Moving into the wider transitional zone, the flow decreases and sedimentation of finer particles occurs. The lacustrine zone is typically near the dam, which is the deepest area of the reservoir, where flow is almost non-existent and nutrients and sediment loads are low (Kalff 2002).

When reservoirs are built, the surrounding river valleys are flooded, submerging the terrestrial vegetation and soil organic matter, thereby providing large amounts of organic matter for decomposition along the bottom (Kelly et al. 1997, Matthews et al. 2005, Huttunen et al. 2002). Over time, high rates of aerobic decomposition can deplete the dissolved oxygen in the water and lead to anoxic conditions in the hypolimnion or near the water-sediment interface (Wetzel 2001). Reservoirs also act as sedimentation basins within a watershed, where the majority of particulates transported in streams are deposited. Most deposition of fine particulate matter occurs in the transitional and lacustrine zones of the reservoir as the rate of flow decreases and the channel widens (Kalff 2002). Increasing available dissolved organic carbon (DOC) or more labile

organic matter in an aquatic ecosystem can increase microbial activity (Wetzel 2001), while sedimentation of fine inorganic particles can inhibit microbial activity (Kaller et al. 2001). The amount of sedimentation occurring in these reservoirs is a major concern for water quality, ecosystem integrity, and the longevity of the reservoirs (St. Louis et al. 2000, Kaller & Hartman 2004).

Sediments will be anaerobic 1-2 cm below the sediment-water interface, and methanogenesis will occur, regardless of water column oxygen. The production of biogenic methane, called methanogenesis, occurs naturally as a byproduct of anaerobic respiration (metabolism in the absence of oxygen) by a group of microorganisms called methanogens (Werne et al. 2002). Methanogens are members of Domain Archaea, require anoxic environments to survive and are vital to the decomposition of organic matter in landfills, wetlands, aquatic sediments and bogs (Woltemate et al. 1984, Jones et al. 2004, Gebert et al. 2003, Lassey et al. 1997).

There are a number of substrates and metabolic pathways that methanogens may utilize for their metabolism. As organic matter decays, electron acceptors (such as oxygen, ferric iron, and nitrate) become depleted, while hydrogen (H_2) and carbon dioxide (CO_2) accumulate. This may stimulate the hydrogenotrophic pathway, which is a chemoautotrophic pathway that uses CO_2 and H_2 as substrates to produce methane. Another common pathway is the acetoclastic pathway, which uses acetate (CH_3COOH) as a substrate. Acetate can accumulate in the sediments from the aerobic decomposition of settling phytoplankton and other organic matter, or can result as a byproduct of fermentation. Acetoclastic methanogenesis is the most primitive and most common

metabolic pathway, even though it is the least energy efficient (Schulz & Conrad 1995, Woltemate et al. 1984).

Hydrogenotrophic methanogenesis: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_20$

Acetoclastic methanogenesis: $CH_3COOH \rightarrow CH_4 + CO_2$

Some of the methane produced by the methanogens in the sediments is released through ebullition into the atmosphere and some methane is consumed by other Archaea bacteria, called methane oxidizing bacteria (MOB). MOB require oxidizing conditions for respiration, but have been found to oxidize sulfate and nitrate in the absence of dissolved oxygen (Thauer & Shima 2006). MOB typically inhabit areas at or near the sediment-water interface of streams, lakes and reservoirs (Chanton 2005). MOB utilize methane as a carbon source and may regulate the amount of biogenic methane that is emitted from the sediments. MOB are also a potential food source for benthic and pelagic consumers (Bastviken et al. 2003). The importance of biogenic methane as a carbon source to a particular organism can be determined in part by looking at the isotopic signatures of the organism and comparing them to the isotopic signatures of other source materials (Jones et al. 2004, Hershey et al. 2005).

Isotopes are alternate forms of elements that have different atomic masses due to their differing number of neutrons. ^{13}C is a stable isotope of carbon that represents about 1.1% of the total carbon on earth and occurs naturally in the atmosphere with a $\delta^{13}C$ value of about -8 ‰ (Ferry 1993). The δ (del) value is defined as $\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1000$ for a specific isotope (^{13}C), where R_{sample} is the ratio of ^{13}C to ^{12}C in a sample and $R_{standard}$ is the ratio of ^{13}C to ^{12}C in the PDB (Pee Dee belemnite) formation

(see Peterson & Fry. 1987). The relative abundance of ^{13}C compared to ^{12}C found in most organisms is fairly low (Langellatto et al. 2005, Chanton 2005). However, fractionation can alter the relative abundance of the two isotopes during reactions (Peterson & Fry. 1987). Because methane is a byproduct of a multi-step decomposition process that enriches the light isotope in the products, it is very depleted in ^{13}C. As a result, the $\delta^{13}C$ value of MOB, methanogens and organisms that consume these bacteria are also very low (Woltemate et al. 1984). The range of $\delta^{13}C$ values in methanogens and MOB is usually between -90 to -50 ‰, but can also be identified by a larger range of $\delta^{13}C$ values (-100 to -21 ‰) depending on the methanogenesis pathway utilized, the initial value of the source material (Chanton. 2005, Eller et al. 2005) and the amount of fractionation during methane oxidation (Venkiteswaran & Schiff 2005, Whiticar 1999, Woltemate et al. 1984, Whiticar & Faber 1985). Previous research found the acetoclastic methanogenesis pathway can fractionate carbon by as much as -42‰ (Krzycki et al. 1987) and methane oxidation by MOB can fractionate carbon by as much as -29.6 ‰ (Barker & Fritz. 1981). These characteristically negative $\delta^{13}C$ values can be used to evaluate the importance of these organisms as food sources to consumers within a food-web. The average change in $\delta^{13}C$ values from a food source to its ocnsumer is usually 0-1 ‰, with the organism typically being more enriched than the food source (Bunn & Boon. 1993). Therefore, organisms consuming methanogens or MOB would have similarly negative $\delta^{13}C$ values.

Chironomid larvae are benthic Diptera that often dominate the soft sediment environments of stream and lake ecosystems. Members of the genus *Chironomus*, in

particular, are very tolerant of low oxygen conditions and other forms of pollution. Benthic organisms, including chironomids, play a central role in processing detritus, decomposing organic matter, and transferring energy through the food web to higher trophic levels. Functionally, most chironomids are considered collector-filterers and/or collector-gatherers (Merrit & Cummins 1996), inhabiting areas at or near the sediment-water interface (Roy et al. 2003, Kelly et al. 2004). Published research on the diet of *Chironomus* rarely indicate complete reliance on any one source (Grey et al. 2004 a refs. therein), and they may feed on organic detritus, algal particulates and bacteria (Merrit & Cummins 1996). Members of the tribe Chironomini, including *Chironomus*, are bright red in color due to high concentrations of hemoglobin, making them more efficient at taking up available oxygen. Capable of withstanding very low dissolved oxygen levels and periods of anoxia, these red chironomids often mine into the sediment or construct chimneys or tubes made of silk and particulates, which are used in both respiration and feeding (Kajan & Frenzel 1999, Stief et al. 2005). This bioturbation of the sediments by the larvae has been shown to actively promote microbial activity (Kelly et al. 2004, Stief et al. 2005) and provide favorable microclimates for methane oxidizing bacteria. Eller et al. (2005) showed evidence of methane/MOB utilization by chironomid larvae, with the $\delta^{13}C$ values of the benthic chironomid larvae depleted by as much as 20 ‰ as compared to surrounding sediment, and a mean del value of -46.9 ± 4.1 ‰. Additionally, research conducted in arctic Alaskan lakes found that a significant portion of the benthic secondary production was based on biogenic methane by way of macroinvertebrate consumption of methanogens and MOB (Hershey et al. 2006). Much of the ecological

research on methanogenesis and methane oxidation has been focused on natural lakes (example, Jones &Grey 2004), wetlands (example, McKenzie et al. 1998) or hydroelectric reservoirs (example, Duchemin et al. 1995) as opposed to warm monomictic or polymictic municipal reservoirs.

My objectives for this study were to determine if methane was a significant resource to *Chironomus* diet in reservoirs in the Piedmont of North Carolina, determine how much methane was being produced and oxidized in these sediments, measure the rates of sedimentation, and determine if a correlation existed between sedimentation and larval chironomid carbon isotope values. I hypothesized that methanogenesis and methane oxidation were occurring in these reservoirs at significant rates, and that methane-derived carbon was a significant resource for benthic chironomids. I further hypothesized that the amount of sedimentation would affect the carbon isotope signature of chironomids, with increasing rates of sedimentation resulting in more positive $\delta^{13}C$ values in the chironomids.

CHAPTER II

MATERIALS AND METHODS

Study sites

This work was conducted in reservoirs serving Greensboro and High Point, two of the three cities in the Piedmont Triad in North Carolina. These reservoirs are located in the headwaters of the Cape Fear River Basin, which is North Carolina's largest river basin located entirely within the state's boundaries. It flows from its headwaters in the north-central piedmont region to its mouth, emptying into the Atlantic Ocean, near Wilmington. The headwaters of the Cape Fear River Basin are found in rural, urban and forested areas. Construction and land development, agricultural practices, stream bank erosion, and storm runoff from urban areas are all major sources of sedimentation and non-point pollution throughout the river basin.

My research locations included Greensboro's three municipal reservoirs, Lake Higgins, Lake Brandt and Lake Townsend, and one of two municipal reservoirs in High Point, High Point City Lake (Figure 1). Lake Higgins is upstream of Lake Brandt, and they are both upstream of Lake Townsend. Oak Hollow Reservoir, the other municipal reservoir for High Point City Lake, is upstream of High Point City Lake. There were two research sites at each reservoir; "basin" sites located downstream of a sedimentation basin or in the lacustrine zone of the reservoir, and "tributary" sites located downstream of tributaries only.

Constructed in 1958, Lake Higgins has one major inlet stream, Brush Creek, and is dammed at Battleground Avenue near Hamburg Mill Road. Its outlet flows directly into Lake Brant. Lake Higgins covers 0.9 km^2 and has a drainage area of 30 km^2. It is surrounded by a buffer of forested area with suburban developments and Bur-Mill Golf Course within an 8 km radius of the buffer. Higgins also serves as a basin for the deposition of sediments and other particulates which are transported in Brush Creek.

Lake Brandt, constructed in 1926, was the first Greensboro municipal reservoir. It covers 3.3 km^2 and drains 151.3 km^2 of land. Beaver Creek, Moore's Creek and Reedy Fork Creek flow into one arm from the west and Horsepen Creek flows into the other arm from the south. Lake Brandt is dammed at Lake Brandt Road and Reedy Fork Creek continues as its outlet, flowing into Lake Townsend. Lake Brandt is also surrounded by a forested buffer, with suburban developments beyond the buffer and Bur-Mill Golf Course bordering a portion of the north arm of the reservoir.

Lake Townsend is the largest of the three Greensboro reservoirs, constructed in 1969, covering an area of 6.2 km^2 and draining 71 km^2 of land. Lake Townsend is fed by a number of low order streams as well as Reedy Fork Creek and Richland Creek, an outlet stream from nearby Lake Jeannette. There is a forested buffer surrounding the reservoir except for a significant portion where the Bryan Park Golf Course borders the southern edge of the main arm of the reservoir. Suburban developments and industrial areas lie beyond the buffer.

High Point City Lake was constructed as a municipal water source in 1935, covering an area of 1.4 km^2. It is currently on the EPA's 303d list for high chlorophyll-a

levels (N.C. Division of Water Quality 2006) and is aerated throughout the main body of the reservoir to prevent thermal stratification and anoxia. Aerators are located about 0.3m above the sediment, in the deepest portions of the reservoir. Due to the aeration system, this reservoir is considered polymictic as it does not freeze over in the winter and does not stably stratify in the summer. The west arm of the lake is fed by the West Fork of the Deep River, which is the outlet stream from Oak Hollow Reservoir, just north-west of High Point City Lake. This outlet stream has been listed on the EPA's 303d list for turbidity. The east arm of the lake is fed by the East Fork of the Deep River, which was listed on the EPA's 303d list for high fecal coliforms, turbidity and impaired ecological integrity.

Sampling and laboratory methods

Field measurements including dissolved oxygen (mg/L), pH, temperature (°C) and chlorophyll-a (μg/L) were taken using a YSI. Secchi depth (m) was taken using a Secchi disk. Field measurements were taken at all sites to assess environmental conditions and thermal profiles of the reservoirs. Sampling for chironomids, seston and sediment cores were conducted once during the summer stratification between May and September in 2006, and once during the winter/spring mixing between January and April in 2007. Methane samples from the water column were taken once from each site.

Chironomid larvae were sampled using an Eckman Grab and sediment was sieved through a 500μm mesh net to separate larvae from sediments. Larvae were placed into containers along with surface water for transport to the laboratory. The majority of

organisms collected from these reservoirs were chironomids in the genus *Chironomus*. These *Chironomus* larvae were retained for use in the study. The reservoir water in each container used to transport the larvae was filtered through 0.47μm glass fiber filters so that the larvae could be contained in the filtered water overnight to allow their guts to clear. After a minimum of 12 hours, the larvae were transferred to weighing dishes and dried in a 60ºC oven for three days. Dried larvae were then homogenized using a mortar and pestle, separated into 1 mg samples and assayed for $\delta^{13}C$ by the Stable Isotope Laboratory at the University of California - Davis. A number of organisms collected were retained for identification to genus, and were identified as *Chironomus*.

Sediment traps were used to collect samples of seston from the water column at each site of all reservoirs. Traps were constructed using four 4"x12" PVC pipes, open at the top end, with four 3"x12" clear plastic mailing tubes placed inside them. Traps were placed one meter above the water-sediment interface and were anchored with a sandbag and buoyed at the surface to maintain water column position. The traps were set out at each site for a minimum of seven days. Upon collection, the contents of each tube were poured into cubitainers for transport back to the laboratory. The water and sediment from the sediment traps was filtered using pre-ashed .47 μm glass fiber filters. One filter from each replicate was used for isotopic analysis and the remaining filters were used for sedimentation rate calculations.

Lake Brandt and High Point City Lake were chosen for core incubation experiments to assess methanogenesis and methane oxidation as they were the most similar reservoirs in age and morphology. Intact sediment cores were collected from both

sites of both reservoirs to evaluate the amount of methanogenesis and methane oxidation occurring in the sediments. Ten cores (54 cm long x 4.8 cm diameter) were taken at each site, with five cores randomly selected as controls and five as treatments. The cores were collected using a KB Corer and a Van Dorn sampler was used to collect water samples and treatment water. Cores were extruded into shorter incubation cores (25 cm long x 4.8 cm diameter) and the hypolimnetic water was removed and replaced with either a 1.5% methyl fluoride (CH_3F) solution in unfiltered hypolimnetic water or a control solution of unfiltered hypolimnetic water. Methyl fluoride has been shown to be a specific inhibitor of methane oxidation at 1.5 % concentration (Oremland & Culbertson, 1992) allowing the methane produced via methanogenesis to accumulate. Stir bars were placed in the cores and overlying water was stirred at 1 rpm, a rate that did not disturb the sediment-water interface. Cores were then incubated at the ambient bottom temperature of the reservoir they were taken from (High Point: 19°C, Brandt mixed: 15°C, Brandt stratified: 20°C). 3 mL samples from of each core were taken at the initial time of the treatment, 14 hours after treatment and 24 hours after treatment. The 3 mL samples were taken using a 10 mL syringe and a 20 gauge needle, and injected into 30-cc serum vials. The serum vials were prepared with 0.2 mL of 1N hydrochloric acid (HCl) and 0.2 mL of mercuric chloride ($HgCl_2$), to inhibit all microbial activity. Air was evacuated from the vials using a vacuum pump, and vials were flushed with 60 mL of He gas using a 60 mL syringe and a 20 gauge needle, with a second needle in the septa to allow the pressure to return to 1 atm. Liquid removed from the incubation cores for sampling was replaced with the respective treatment water to ensure no headspace remained in the cores. Serum vials

were also stored at the ambient bottom temperature of the reservoir until they could be analyzed using a gas chromatograph. 3mL gas samples were taken from the serum vials after being vigorously shaken to equilibrate the gases in the vial, and injected into a Shimadzu GC8A gas chromatograph equipped with a flame ionization detector. The water samples taken at each site were placed in 40 mL amber vials containing 0.2 mL mercuric chloride ($HgCl_2$) and 8mLs from each sample were transferred into a pre-evacuated 12 mL exetainer, leaving 4 mL of headspace. The methane gas in the exetainers was then analyzed by the University of California Davis Isotope Laboratory using isotope ratio mass spectrometry.

Sedimentation rates were calculated from Lake Higgins, Lake Townsend and Lake Brandt, but High Point City Lake was excluded from sedimentation rate calculations due to the excessive resuspension of sedimenting material in the aerated portions of the reservoir. All 0.47 μm glass fiber filters used for sedimentation rate analyses (ashed dry mass (ADM) and ash free dry mass (AFDM)) were pre-ashed at 500 ºC for 2 hours and weighed before use. The amount of inorganic (ADM) and organic (AFDM) sedimentation was measured from each filter, taking into account the number of days each trap was set out.

Data analysis tools

Paired-sample t-tests were used to compare the $\delta^{13}C$ values of (1) *Chironomus* to the seston at each site, (2) the value of *Chironomus* during stratification vs. during mixing (excluding High Point City Lake) and (3) the value of *Chironomus* at basin sites vs.

tributary sites. A two-source mixing model was used to calculate the proportion of seston and methane in the diet of *Chironomus*. I used the following equation for the mixing model calculations (Bunn & Boon 1993): $\%(A) = (C - f_1 - B)/(A - f_2 - B)$, where f_1 is the fractionation factor (‰) for the consumer, C is the del value (‰) of the consumer, f_2 is the fractionation factor for MOB and A and B are the del values (‰) of the two food sources (MOB and seston). A range of fractionation values were used for the fractionation of methane by MOB (1‰, 16‰, 30‰) as a wide range of values has been reported in the literature (Venkiteswaran & Schiff 2005, Whiticar 1999, Whiticar & Faber 1986). An estimated fractionation value of 1‰ was used for the fractionation by *Chironomus* (Peterson & Fry 1987). Two-way ANOVAs were used to analyze *Chironomus* $\delta^{13}C$ values by reservoir and site type during mixing and stratification. Paired-sample t-tests were also used to compare the rates of sedimentation between thermal profiles (stratification and mixing) and between sites within each reservoir. Simple linear regression analysis was used to analyze the relationship between the $\delta^{13}C$ values of the chironomids and (1) total sedimentation rate, (2) inorganic sedimentation rate and (3) organic sedimentation rate. Simple linear regression analysis was also used to analyze the relationship between the percent organic matter in the sediment and the sedimentation rates (total, inorganic and organic). All significant relationships reported are those with a p-value less than .05. Standard deviations will be reported for all average values given (average ± SD).

CHAPTER III

RESULTS

Sedimentation

The sedimentation rates calculated for all reservoirs were quite variable by site and thermal profile, with no significant difference between basin and tributary sites overall (Figure 2). However, in Lake Brandt during stratification, the tributary site had a significantly higher rate of sedimentation than the basin site ($p < .001$, $t = -22.806$, d.f. = 3, Table 1), with the average sedimentation rate at the tributary site 3.3 times greater than the basin site.

The percent organic matter of the sediment trap material was similar during stratification (21.7 ± 5.6%) and mixing events (24.4 ± 5.3%, mean ± SD; Figure 3). However, during stratification, the percent organic matter was significantly greater in the basin sites of Lakes Brandt and Townsend than in the tributary sites (Brandt: $p = .007$, $t = 6.547$, d.f. = 3, Townsend: $p = .002$, $t = 9.482$, d.f. = 3), a pattern that was not observed during mixing or in Lake Higgins (Figure 3). Simple linear regression analysis showed a significant negative correlation between total sedimentation rate and the percent organic matter during stratification ($p < .001$, $R^2 = .729$) (Figure 4) and during mixing ($p = .004$, $R^2 = .451$) (Figure 5). While reservoirs were mixing, a significant positive linear relationship was found between the $\delta^{13}C$ values of *Chironomus* and the rate of inorganic sedimentation ($p = .034$, $R^2 = .215$) (Figure 6).

Methanogenesis and methane oxidation

Rates of methanogenesis were substantial at both sites of Lake Brandt during stratification and at both sites in High Point City Lake (568 – 1515 $mg/m^2/d$; Table 2). Rates of methogenesis were considerably lower at both sites of Lake Brandt during mixing (approximately 25 $mg/m^2/d$) (Table 2). Methane oxidation rates (the arithmetic difference between treated and control cores) were also substantial in the tributary site of Lake Brandt during stratification (1,101 ± 235 $mg/m^2/day$) and in the tributary site of High Point City Lake (989 ± 284) (Table 2), but quite variable across other sites. Overall, methane oxidation ranged from 15 – 92 % of that produced, and the percent oxidized was not correlated to the rate of methanogenesis.

Average concentrations of methane over time in the incubated cores from High Point City Lake and Lake Brandt are shown in Figure 7. Lake Brandt had significantly more methane after 24 hours in the treated cores during stratification than mixing ($p = .003$, $t = 4.895$, d.f. =6) (Figure 7). With the exception of the basin site of High Point City Lake, methane concentrations in control cores remained fairly constant over the 24 hour incubation period, while methane concentrations in CH_3F treated cores increased through time (Figure 7).

Carbon stable isotope values

Overall, *Chironomus* was significantly depleted in ^{13}C compared to seston ($p < .001$, d.f. = 15, $t = -6.891$, average $\delta^{13}C$ shift = -5.3‰) (Table 3). Comparing each site and sampling event individually, *Chironomus* was significantly more depleted in ^{13}C than

seston during the summer stratification in both sites of Lake Higgins and the basin sites of Lake Townsend and High Point City Lake (Figure 8A). During the winter/spring mixing, *Chironomus* was significantly more depleted in ^{13}C than seston at both sites of each reservoir (Figure 8B). Stratification did not have a significant effect on the $\delta^{13}C$ values of *Chironomus* overall ($p = .234$, $t = -1.219$, d.f. = 26); the average $\delta^{13}C$ value of *Chironomus* during stratification (-36.3 ± 4.6‰) was similar to the average value during mixing (-36.5 ± 2.3‰) (Table 3). Seston values for both thermal profiles were more enriched than *Chironomus*, averaging -30.4 ± 0.7‰ and -31.3 ± 2.0‰ during stratification and mixing respectively (Table 3). A 2-way ANOVA of reservoir and site type on *Chironomus* $\delta^{13}C$ values during the winter/spring mixing showed a significant site type effect and a significant reservoir effect with 98% of the variation explained by the model ($p < .001$, $F_{7,12} = 82.735$). No significant site type or reservoir effects were found during stratification. Paired sample t-tests showed significant differences between the average *Chironomus* $\delta^{13}C$ values in basin sites vs. tributary sites during stratification in Lake Higgins and Lake Townsend, and in all reservoirs during mixing (Figures 8A and 8B).

The methane $\delta^{13}C$ values measured from the water samples taken at each site averaged as low as -72.0 ± 1.0‰ in Lake Townsend's tributary site and as high as -32.8 ± 36.1‰ in Lake Higgins tributary site (Table 4). Methane $\delta^{13}C$ values were significantly more negative than *Chironomus* $\delta^{13}C$ values in Lake Brandt and the basin site of High Point City Lake. The two-source mixing models estimated the proportion of *Chironomus* diet from methane was between 4 and 89%, with the average percent contribution from

all sites and reservoirs between 10 ± 5% and 36 ± 28% (Table 4). *Chironomus* from High Point City Lake had almost identical average $\delta^{13}C$ values as methane in the tributary site, with 17 - 89% of the diet contributed by methane. Also, there was no significant difference in $\delta^{13}C$ values between the methane and *Chironomus* from either site of Lake Higgins, with 16 - 39% and 8 - 65% of the diet due to methane in the basin and tributary sites respectively (Table 4). Lake Townsend's tributary site during stratification had the lowest percent contribution (4 - 7%) from biogenic methane via MOB (Table 4).

CHAPTER IV

DISCUSSION

Studies of tropical reservoirs indicate that methane emissions may be significant anthropogenic sources to the atmospheric methane pool (St. Louis et al. 2000). Some recent studies in temperate reservoirs indicate that methane-derived carbon may also be important in aquatic food webs, especially via ingestion of MOB by *Chironomus* (e. g., Grey et al. 2004, Jones and Grey 2004). Here, we discuss results from 4 warm temperate reservoirs which indicate that, although variable, production of methane and utilization of methane-derived carbon can be very high, and consider factors that affect variability in those processes in these reservoirs.

Sedimentation

Major sources of sediment to streams and reservoirs include channel sediment from bed and bank erosion and non-channel sediment from exposed soils during construction or other anthropogenic activities (Wood & Armitage 1997 and refs. within). The reservoirs we studied were in urban and suburban areas with agriculture and/or construction occurring within the watersheds. The rates of sedimentation we calculated from sediment traps were variable by site, reservoir and sampling time. Variable sedimentation rates are common, and have been reported, in urban areas where increases

in the volume and speed of runoff during rain events can cause sharp increases in sediment load (Gray 2004, Wood & Armitage 1997).

Data from sediment traps set out during stratification in late-August to early-September showed that the basin site of Lake Higgins had the highest rate of sedimentation when compared to the basin sites of Lake Brandt and Lake Townsend during that same time period (Figure 2). This may be because the basin site of Lake Higgins is located in the same arm of the reservoir as the tributary site, rather than in a separate arm, with no reservoir upstream (Figure 1). Upstream reservoirs can act as sedimentation basins and retain a significant portion of the sediment that would otherwise travel downstream. Because there is no reservoir upstream of Lake Higgins, more sedimentation may have reached both sites of this reservoir.

Tributary sites had no upstream sedimentation basins ; therefore, variability in sedimentation rates in tributary sites could be due, in part, to sediment transported to the reservoirs during rainfall events (Krishnaswamy et al. 2006, Gray 2004). During stratification, Lake Brandt's tributary site had the highest average rate of sedimentation when compared to all other sites (Figure 2). This high rate, recorded in late August, was likely due to a rain event that occurred while that trap was deployed.

The significant negative relationship between the rate of sedimentation and the percent organic matter of the sedimentation was observed during both mixing and stratification in Lake Brandt, Lake Higgins and Lake Townsend. Therefore, as the rate of sedimentation increased in the reservoirs, a smaller percentage of it was comprised of organic matter. This indicates contributions of primarily inorganic sediment from the

watershed, most likely linked to rain events (Gray 2004). Although the percent of organic matter in sedimentation has seldom been reported in the literature, the amounts of organic material in the top layers of the sediment have been reported in order to describe benthic sediments and communities (Wood & Armitage 1997 and refs. within).

The positive response of *Chironomus* $\delta^{13}C$ values to increased inorganic sedimentation during mixing suggests that the increased inorganic sedimentation caused a proportional shift in the diet of *Chironomus* away from biogenic methane. Although only 22% of the variation was explained by the linear regression model, it was a statistically significant relationship and should be further investigated. It's possible that MOB could have become a less-available food source to *Chironomus* if the tubes and chimneys occupied by them were buried by the additional sediments, although our study was not designed to test this hypothesis.

Methanogenesis and methane oxidation

The high rates of methanogenesis in treated cores from High Point City Lake on October 10, 2007 occurred even though aerators were mixing the reservoir. Although High Point City Lake is constantly being mixed, the aerators do not sit directly on the sediment surface and only occupy a linear section of the deepest portions of the lacustrine and transitional zones (Figure 1). This may allow for some areas of the reservoir to weakly stratify for short periods of time, especially during the summer, or allow for lower DO levels along the sediment-water interface. High Point City Lake had higher rates of methanogenesis but also lower rates of methane oxidation than Lake Brandt. The

average net methane emission rate (methane produced via methanogenesis but not consumed via oxidation) from the sediments of High Point City Lake (839.6 ± 584.4 $mg/m^2/day$) was much higher than average flux rates estimated from tropical reservoirs (300 $mg/m^2/day$) by St. Louis et al. (2000). In addition, considerably high rates of methanogenesis were recorded (up to 2,585.5 $mg/m^2/day$) from High Point City Lake's basin site. One possible reason for the high levels of methanogenesis in High Point City Lake could be an abundance of DOC or higher quality DOC in the top layers of the sediment, although DOC quality and quantity were not measured in this study. However, we do know that High Point City Lake and its tributaries were listed on the EPA's 303d list as impaired due to high algal levels (N.C. Division of Water Quality 2006), which could have contributed organic matter to acetoclastic methanogens through the decomposition of the algae by other microbes.

Lake Brandt also produced more methane than was consumed by MOB, with an average net methane emission rate from the sediments of cores taken on April 9, 2007 during mixing (16.7 ± 6.3 $mg/m^2/day$) similar to the average flux rate from north temperate reservoirs (20 $mg/m^2/day$) estimated by St. Louis et al. (2000). From the cores taken during stratification on May 30, 2007 in Lake Brandt, the average net methane emission rate (147.8 ± 142.2 $mg/m^2/day$) was much higher than the estimated average flux rate from north temperate reservoirs. These methane emissions from the sediments can migrate up through the sediments and water column where they may eventually be released to the atmosphere through diffusion, or they can be released directly to the atmosphere from the sediments via ebullition. The amounts of net methane emissions

observed from the sediments of these warm temperate reservoirs are of particular concern when considering anthropogenic greenhouse gas emissions and their impact on the global climate (Matthews et al. 2005, Kelly et al. 1997, St. Louis et al. 2000, Venkiteswaran & Schiff 2005).

Other factors, such as temperature, may have had an impact on the rates of methanogenesis and methane oxidation we observed (Valentine 2002, Venkiteswaran & Schiff 2005, McKenzie et al. 1998). McKenzie et al. (1998) found methane production rates could triple with a 10°C temperature increase in flooded boreal reservoirs, although this increase was greater in the 4 – 15 ºC range than the 15 - 25 ºC range and was observed primarily in floating peat islands. They attributed the increase in methane production to an increase in the metabolic rate of methanogens or fermentors within the floating peat islands. Similarly, Venkiteswaran & Schiff (2005) observed an increase in methane oxidation rates with an increase in temperature in the Experimental Lakes Area Reservoir Project (ELARP) after flooding boreal forest wetlands in northwestern Ontario (incubation temperature range: 18 - 30ºC). Incubation temperatures of sediment cores taken from Lake Brandt during mixing and stratification were 15°C and 20°C respectively, and the incubation temperature of cores from High Point City Lake was 19°C. Therefore, it is possible that the lower rates of methanogenesis in Lake Brandt during mixing, compared to rates during stratification, were due, in part, to the lower incubation temperature.

Increases in methane concentrations from the control cores, similar to those observed in the treated cores, were observed in cores taken from the basin site of High

Point City Lake. It is unclear why this occurred, but one possibility is that MOB were not as abundant in the basin site sediment cores. MOB are not ubiquitous in freshwater sediments and their distribution can depend on the distribution of chironomid tubes and chimneys (Kajan & Frenzel 1999, Stief et al. 2005) or the availability of oxidizing substrates (Matthews et al. 2005, Kelly et al. 1997).

Carbon stable isotope values

Isotope analysis and mixing model results provide evidence that *Chironomus* do consume biogenic methane via MOB in these reservoirs. The average change in $\delta^{13}C$ values from an organism to its food source is usually between 0-1‰ (Peterson & Fry 1987), with the organism typically being enriched in ^{13}C compared to the food source. *Chironomus* ^{13}C values were depleted by 5.3‰ compared to seston, which provides strong evidence that *Chironomus* are consuming isotopically lighter carbon sources in addition to seston.

From the $\delta^{13}C$ values of the methane (Table 4), it is clear that *Chironomus* are not solely consuming methane via MOB. As previous studies have shown, *Chironomus* rarely utilize one carbon source exclusively (Grey et al. 2004 a, Bunn & Boon 1993). Therefore, the two-source mixing model was utilized to determine the proportion of the *Chironomus* diet from each substance. However, as previously stated, the fractionation factors observed for MOB in the literature are quite variable (1-31‰) and conclusions regarding fractionation are contradictory in some cases; Coleman et al. (1981) found MOB fractionation increased with an increase in temperature, while Tyler et al. (1994)

found changes in MOB fractionation had little dependence on temperature with only a slight decrease in fractionation with an increase in temperature. However, if increasing temperatures did significantly decrease MOB fractionation, then the fractionation amount to be used for data collected during mixing should be higher than the amount during stratification, and vice versa. The average MOB fractionation amount observed in temperate forest soils was 17‰ (Tyler et al. 1994), in the ELARP ponds it was 19.9‰ and in the FLooded Upland Dynamics EXperiment reservoirs it was 13.7‰ (see Venkiteswaran & Schiff 2005). However, natural observations compiled by Whiticar (1999) found fractionation amounts of less than 5‰ (Steudler & Whiticar 1998) and as high as 27‰ (King et al. 1989), whereas laboratory experiments had slightly higher fractionation amounts, with amounts between 5 and 31‰ (Barker & Fritz 1981), 13 and 25‰ (Coleman et al. 1981) and 7 and 27‰ (Zyakun et al. 1984). The variability in MOB fractionation values may be due to environmental conditions such as temperature or salinity of the surrounding water, methane supply, sediment organic content, sediment porosity or biochemical characteristics of the MOB. There is no consensus regarding isotope fractionation of methane by MOB, therefore, we included a range of fractionation amounts to account for this variability (Venkiteswaran & Schiff 2005, Whiticar 1999, Woltemate et al. 1984, Whiticar & Faber 1985).

High Point City Lake had the highest proportion of *Chironomus* diet due to biogenic methane, comprising up to 89% of the diet in the tributary site (Table 4). Lake Townsend's tributary site had the lowest proportion, with proportions as low as 4% of the diet due to biogenic methane. Research in arctic lakes utilizing a 1‰ fractionation

amount for MOB (Hershey et al. 2005) estimated methanogen/MOB assemblages contributed (17 – 37%) to *Chironomus* tissue and research in northern temperate lakes had estimated contributions of (3 – 58%) (Kelly et al. 2004) and (17 – 29%) (Eller et al. 2005). While these comparisons are from natural lakes and not reservoirs, they still provide a meaningful comparison with regard to freshwater ecosystems. They also allow us to see that the average percent contribution from biogenic methane in our reservoirs, using a 1‰ fractionation factor for MOB (36 ± 28%), is substantial (Table 3). Even using the highest fractionation amount (30‰) in the mixing model, *Chironomus* still had between 4 – 17% of their diet based on methane via MOB.

Chironomus $\delta^{13}C$ values were variable by site, reservoir and sampling time. While collecting samples in the field, various sizes of *Chironomus* were collected, with pupating larvae found at more than one time and location. Although these pupating larvae were not included in isotope analysis, they did indicate that different developmental stages were present simultaneously in these reservoirs. This may have contributed to some of the variation in *Chironomus* isotope values between sites and reservoirs, as larger individuals dominate isotopic signatures in homogenized samples due to their mass.

Because High Point City Lake's tributary site had the highest proportion of *Chironomus* diet due to methane, the highest rate of methanogenesis, and was listed as impaired by the EPA due to algae, it is possible that nutrient input could be linked to both methane production and the incorporation of that methane into the reservoir food web. High Point City Lake's basin site also had a fairly low percentage of methane oxidation

(15%) at the basin site, which left a large amount of methane to be emitted to the atmosphere. We suggest that managing the input of nutrients into reservoirs and lakes could not only help alleviate water quality issues, but reduce the amount of greenhouse gases being emitted to the atmosphere.

CHAPTER V

CONCLUSIONS

Results of this study indicate that rates of methanogenesis and methane oxidation are substantial in some reservoirs, *Chironomus* consume a substantial amount of biogenic methane via MOB, and sedimentation is correlated with *Chironomus* $\delta^{13}C$ values in NC Piedmont reservoirs. Also, these reservoirs may be significant contributors of methane to the atmosphere. Sedimentation had an impact on *Chironomus* carbon sources, with increases in inorganic sedimentation rate associated with higher $\delta^{13}C$ values for *Chironomus* and decreased organic composition of the sediments.

Methane production, oxidation, and food web utilization processes are not well studied in warm temperate reservoirs; therefore, the findings from this research should be useful for further research on reservoirs in similar latitudes. The effect of land use practices on sedimentation, and the consequences of those practices for MOB and food web utilization of methane-derived carbon need further investigation. In addition, further understanding of the impact of nutrient loading on methane emissions in reservoirs is needed, as our estimates indicate that in the most eutrophic reservoir, methane emissions from sediments were very high. As the number of reservoirs continues to grow with the rising demand for water in populated regions, it will become increasingly important to better understand methane processes within them.

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Table 1. Average total and inorganic sedimentation rates and percent organic matter.

Site	Sampling Date	Total (mg/m2/day)	Inorganic (mg/m2/day)	Percent Organic (%)
Townsend Tributary	Stratified '06	0.252 ± .02	0.186 ± .01	26.2 ± 1*
Townsend Basin	Stratified '06	0.242 ± .02	0.171 ± .02	29.3 ± 1*
Brandt Tributary	Stratified '06	1.398 ± .08*	1.157 ± .08*	17.2 ± 2*
Brandt Basin	Stratified '06	0.420 ± .04*	0.315 ± .03*	25.0 ± 1*
Higgins Tributary	Stratified '06	0.800 ± .31	0.673 ± .27	15.9 ± 2
Higgins Basin	Stratified '06	0.992 ± .07	0.834 ± .06	16.0 ± 1
Townsend Tributary	Mixed '07	0.657	0.529	19.5
Townsend Basin	Mixed '07	0.372	0.29	22.0
Brandt Tributary	Mixed '07	0.257 ± .02	0.198 ± .02	23.0 ± 1
Brandt Basin	Mixed '07	0.315 ± .11	0.246 ± .08	21.9 ± 0
Higgins Tributary	Mixed '07	1.302 ± 1.17	1.069 ± 1.03	17.9 ± 8
Higgins Basin	Mixed '07	0.188 ± .02	0.128 ± .01	31.9 ± 0

Stratified samples were taken in September (2006) and mixed samples were taken between January and March (2007). Data given are average ± standard deviation. Sample size (n) was 4 except for Lake Townsend during mixing, which had only data point for each site. Significant differences between basin and tributary sites are indicated with (*).

Table 2. Core incubation data for Lake Brandt and High Point City Lake after 24 hrs. incubation.

Site	Sampling Date	Methanogenesis ($mg/m^2/day$)	Methane Oxidation ($mg/m^2/day$)	Net Methane emissions ($mg/m^2/day$)	% Methane Oxidized
High Point Basin	10-Oct-06	1515 ± 306	221 ± 306	1294 ± 506	15
High Point Tributary	10-Oct-06	1465 ± 283	989 ± 284	476 ± 346	67 ± 23
Brandt Basin	9-Apr-07	25 ± 12	4 ± 6	21 ± 6	16
Brandt Tributary	9-Apr-07	25 ± 9	13 ± 6	12 ± 3	51
Brandt Basin	30-May-07	568 ± 316	381 ± 132	188 ± 185	67 ± 44
Brandt Tributary	30-May-07	1196 ± 280	1101 ± 235	95 ± 44	92 ± 3

All data are given in average ± standard deviation. Standard deviations for average % CH_4 oxidized could not be calculated for Lake Brandt during mixing or for High Point City Lake basin due to unequal sample sizes. Rates of methanogenesis are based on values from treated cores, methane oxidation rates are from the arithmetic difference between control and treated cores, and net methane emissions are based on values from the control cores.

Table 3. Average $\delta^{13}C$ values ± SD for *Chironomus* and seston

Site	Sampling Time	*Chironomus* $\delta^{13}C$ (‰)	n	Seston $\delta^{13}C$ (‰)	n
Townsend Tributary	summer 2006	-33.3 ± 1.0	2	-31.2 ± 0.05	3
Townsend Basin	summer 2006	-39.6 *	1	-31.1 ± 0.09	3
Brandt Tributary	summer 2006	-35.5 ± 4.7	3	-29.7 ± 0.03	3
Brandt Basin	summer 2006	-31.8 ± 2.8	2	-30.5 ± 0.04	3
Higgins Tributary	summer 2006	-31.4 ± 0.08 *	4	-29.9 ± 0.03	3
Higgins Basin	summer 2006	-37.6 ± 1.2 *	3	-31.0 ± 0.13	3
High Point City Tributary	summer 2006	-39.1 ± 5.0	3	-29.4 ± 0.33	3
High Point City Basin	summer 2006	-40.1 ± 4.6 *	2	-30.2 ± 0.21	3
Townsend Tributary	winter/spring 2007	-35.4 ± 0.48 *	4	-27.8 ± 0.12	4
Townsend Basin	winter/spring 2007	-31.7 ± 0.16 *	3	-28.5 ± 0.07	4
Brandt Tributary	winter/spring 2007	-39.6 ± 0.44 *	6	-31.7 ± 0.25	4
Brandt Basin	winter/spring 2007	-35.2 ± 0.15 *	6	-32.3 ± 0.19	4
Higgins Tributary	winter/spring 2007	-39.0 ± 0.26 *	4	-31.3 ± 1.3	4
Higgins Basin	winter/spring 2007	-35.9 ± 0.73 *	4	-32.6 ± 0.14	4
High Point City Tributary	winter/spring 2007	-37.8 ± 0.37 *	4	-32.8 ± 0.20	4
High Point City Basin	winter/spring 2007	-35.0 ± 0.10 *	4	-33.6 ± 0.05	4

Significant differences between *Chironomus* and seston $\delta^{13}C$ values are indicated with (*). Summer samples were taken between May and September and winter/spring samples were taken between January and April.

Table 4. Average $\delta^{13}C$ values for *Chironomus* and methane and mixing model results for the proportion of *Chironomus* diet due to methane. A range of fractionation values were used for MOB fractionation of methane.

Site	Sampling Date	Chironomid $\delta^{13}C$ (‰)	Methane $\delta^{13}C$ (‰)	Proportion due to CH_4 (1‰)	Proportion due to CH_4 (16‰)	Proportion due to CH_4 (30‰)
Townsend Tributary	stratified 2006/2007	-33.3 ± 1.0 *	-72.0 ± 1.0	0.07	0.05	0.04
Townsend Basin	stratified 2006/2007	-39.6 *	-69.9 ± 3.8	0.24	0.17	0.14
Brandt Tributary	mixed 2007	-39.6 ± 0.4 *	-69.7 ± 0.4	0.23	0.16	0.13
Brandt Basin	mixed 2007	-35.2 ± 0.2 *	-70.7 ± 0.3	0.10	0.07	0.06
Higgins Tributary	stratified 2006/2007	-31.4 ± 0.1	-32.8 ± 36.1	0.65	0.14	0.08
Higgins Basin	stratified 2006/2007	-37.6 ± 1.2	-49.6 ± 26.6	0.39	0.22	0.16
High Point Tributary	mixed 2007	-37.8 ± 0.4	-38.5 ± 1.5	0.89	0.28	0.17
High Point Basin	mixed 2007	-35.0 ± 0.1 *	-40.8 ± 1.9	0.30	0.10	0.07
Avg.				0.36	0.15	0.10
SD				0.28	0.07	0.05

Model assumes a 1‰ fractionation for *Chironomus*. Stratified samples were taken between May and September and mixed samples were taken between January and April. The range of fractionation values (1-30‰) accounts for variability in MOB fractionation reported in the literature (see review by Whiticar 1999). $\delta^{13}C$ values given are average ± standard deviation. Significant differences between *Chironomus* and methane are indicated with (*).

APPENDIX B. FIGURES

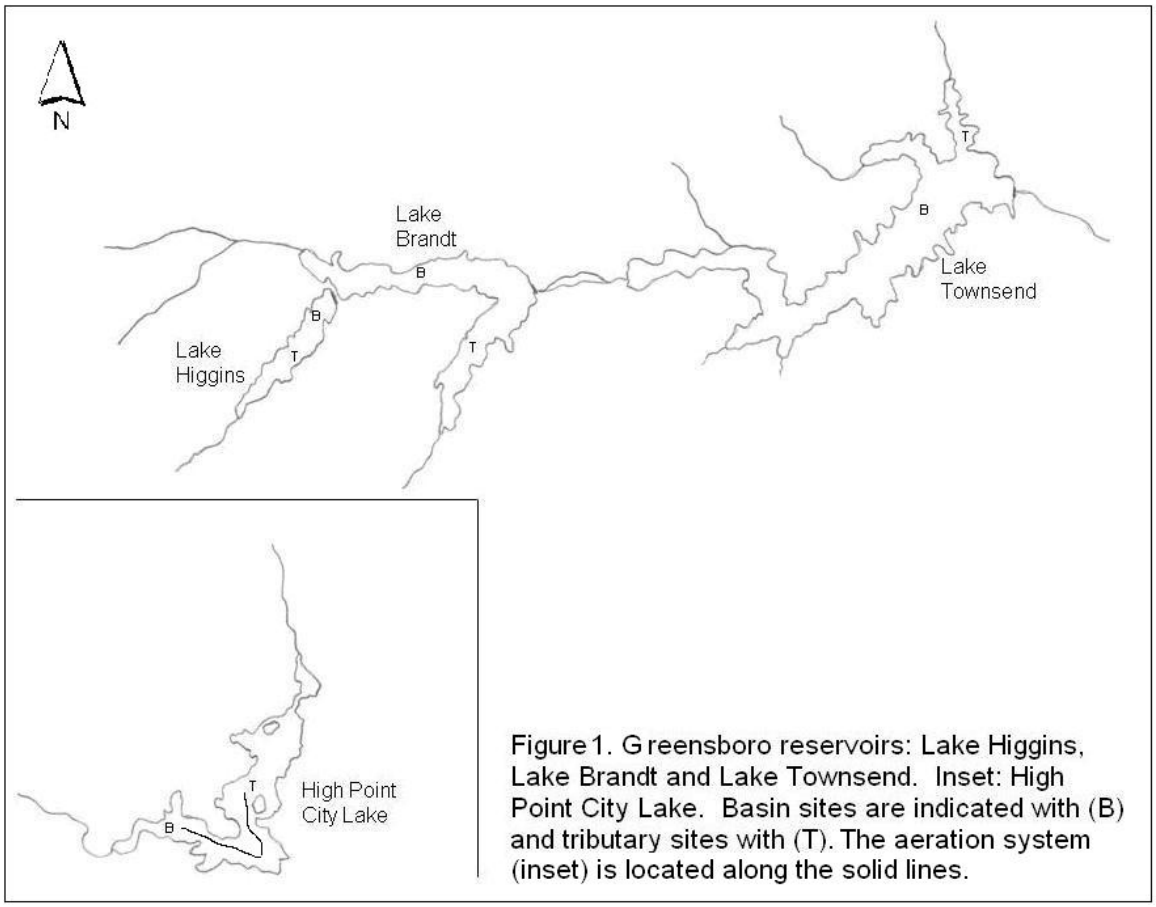

Figure 1. Greensboro reservoirs: Lake Higgins, Lake Brandt and Lake Townsend. Inset: High Point City Lake. Basin sites are indicated with (B) and tributary sites with (T). The aeration system (inset) is located along the solid lines.

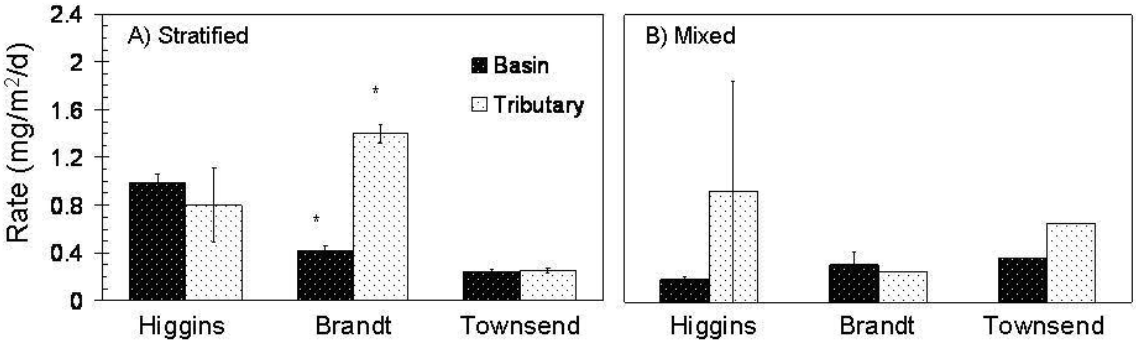

Figure 2. Sedimentation rates (mg/m^2/day) during (A) stratification in 2006 and (B) mixing in 2007. Significant differences between sites are indicated with (*). Townsend had only one data point for each site in 2007.

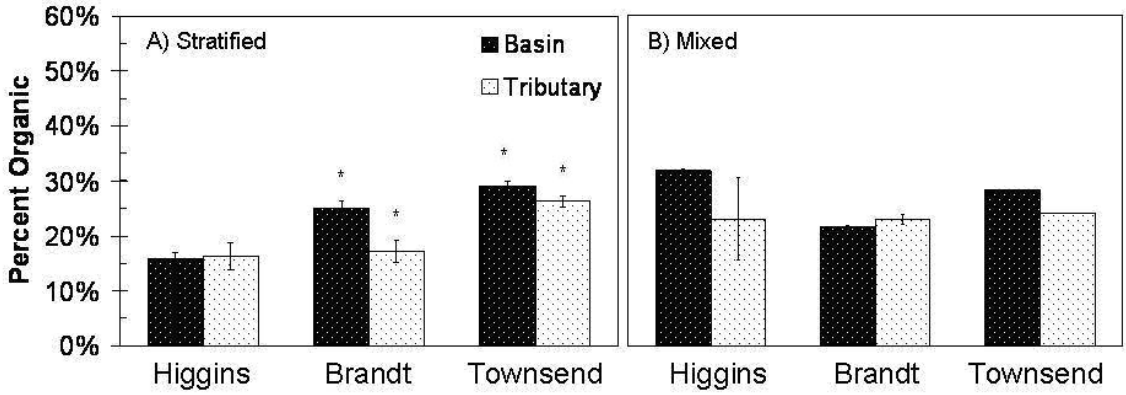

Figure 3. Percent of sedimentation represented by organic matter for both sites of each reservoir measured in (A) 2006 and (B) 2007. Significant differences between sites are indicated with (*). Townsend had only one data point for each site in 2007.

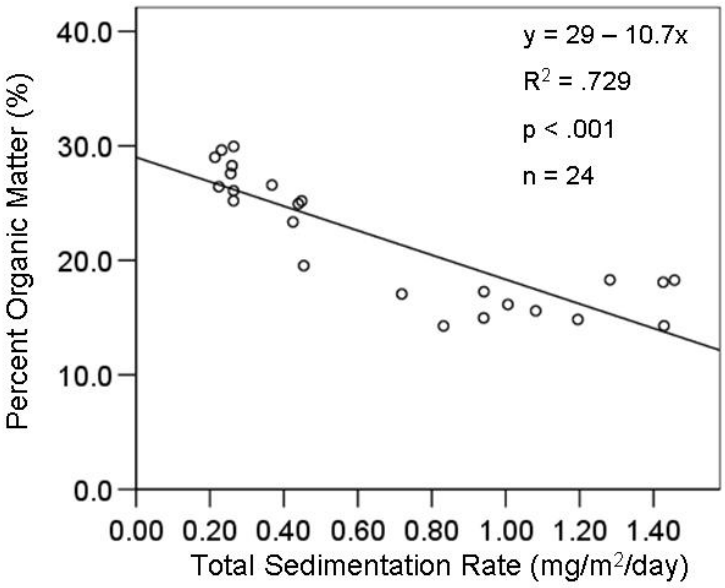

Figure 4. Simple linear regression of percent organic matter and total sedimentation rate during the summer stratification (May – Sept. 2006) in Brandt, Higgins and Townsend.

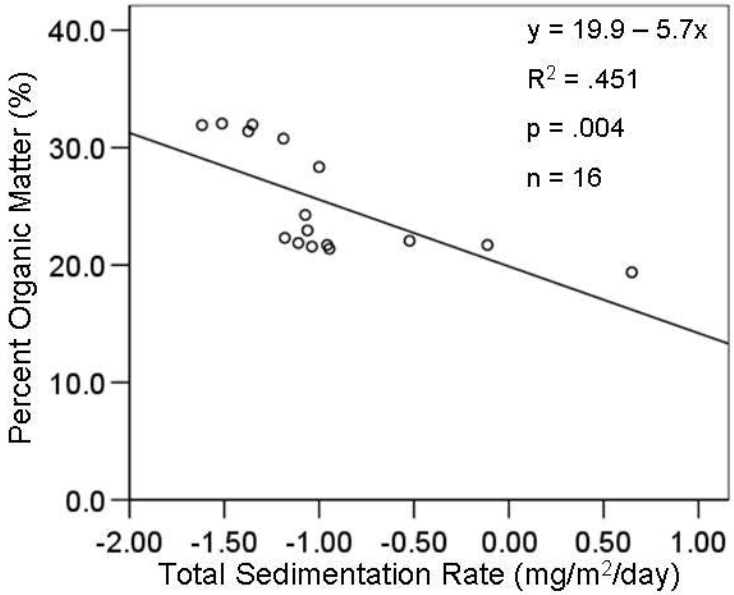

Figure 5. Simple linear regression of percent organic matter and the logit transformed total sedimentation rate during the winter/spring mixing (Jan. - Apr. 2007) in Brandt, Higgins and Townsend.

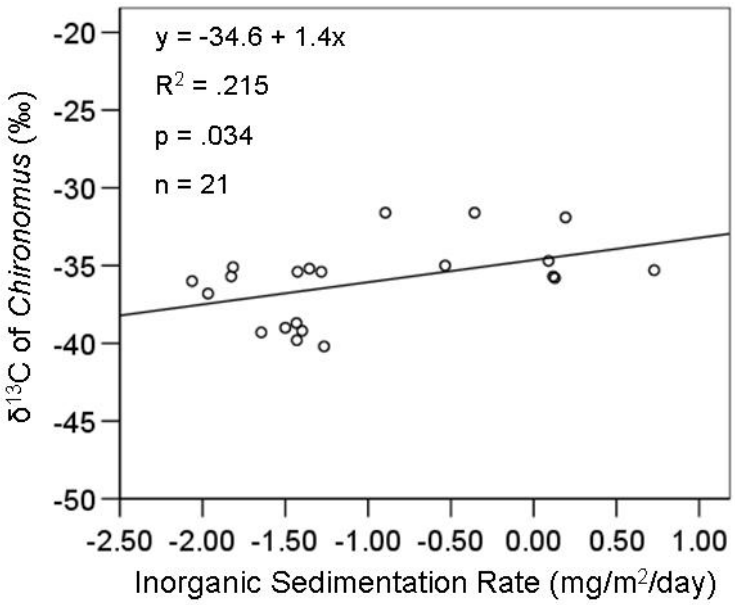

Figure 6. Simple linear regression of *Chironomus* values and the logit transformed inorganic sedimentation rates in Brandt, Townsend and Higgins during the winter/spring mixing (Jan. – Apr. 2007).

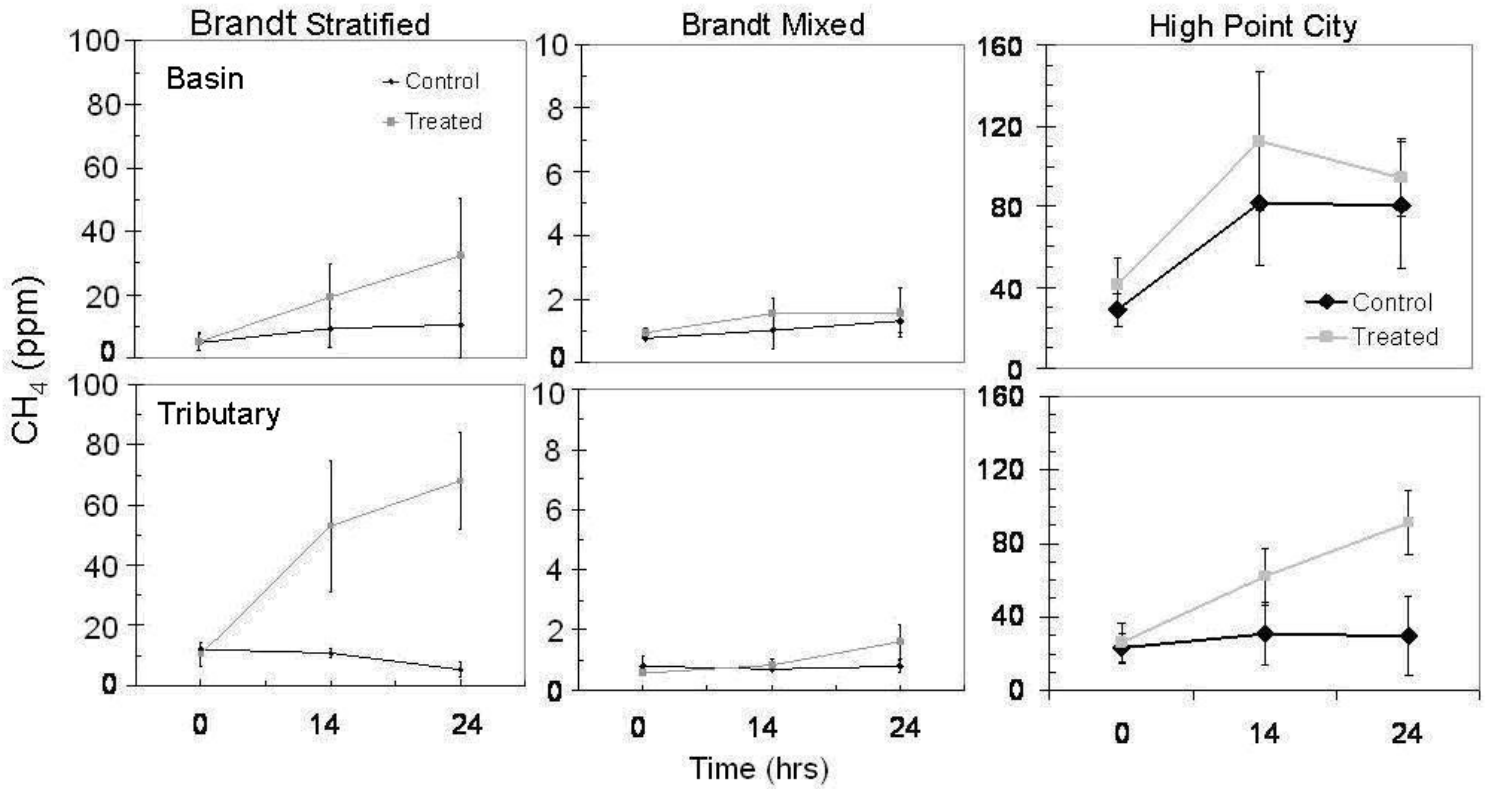

Figure 7. Methane concentrations in treated (methanogenesis) and control cores (methanogenesis and methane oxidation) taken from the basin and tributary sites of Lake Brandt during stratification (5/30/07) and during mixing (4/9/07) and from High Point City Lake (10/10/06). Methane oxidation concentrations were calculated from the difference between treated and control values. Net methane concentrations are represented by the control values.

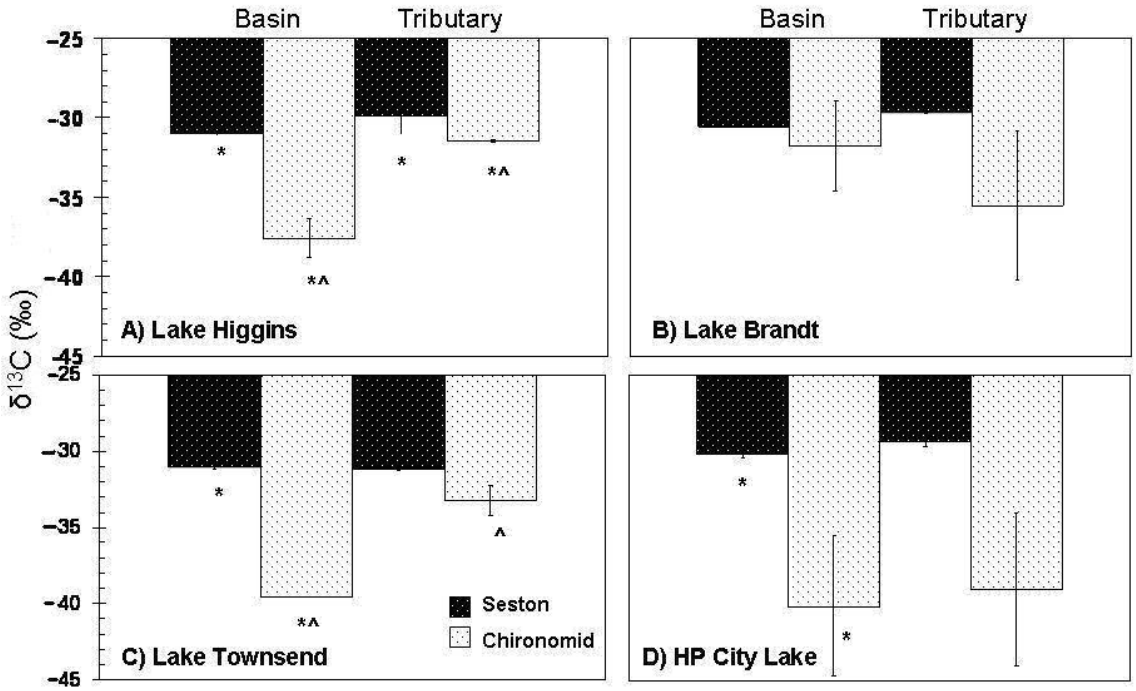

Figure 8A. $\delta^{13}C$ values for seston and chironomid larvae in the basin and tributary sites for each reservoir during the summer stratification in 2006. Significant differences between seston and chironomid larvae are indicated with (*) and significant differences between basin sites and tributary sites are indicated with (^). Statistics for significant differences between chironomids and seston are given below:
Higgins Basin: $p = .013$, $t = -8.614$, d.f. = 2
Higgins Tributary: $p < .001$, $t = -51.754$, d.f. = 2
Townsend Basin: $p < .001$, $t = -168.641$, d.f. = 2
High Point City Basin: $p = .035$, $t = -5.198$, d.f. = 2

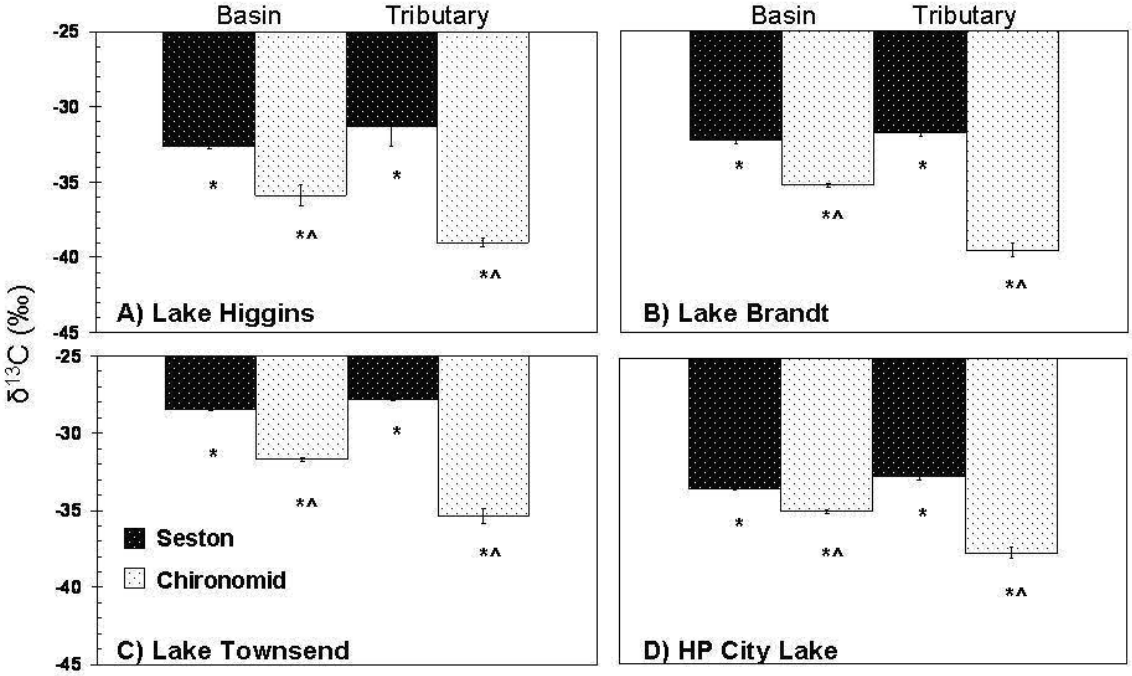

Figure 8B. $\delta^{13}C$ values for seston and chironomid larvae in the basin and tributary sites for each reservoir during the winter mixing in 2007. Significant differences between seston and chironomid larvae are indicated with (*) and significant differences between basin sites and tributary sites are indicated with (^). Statistics for significant differences between chironomids and seston are given below:

Higgins Basin: $p = .002$, $t = -9.518$, d.f. = 3
Higgins Tributary: $p = .001$, $t = -11.994$, d.f. = 3
Brandt Basin: $p < .001$, $t = -17.540$, d.f. = 3
Brandt Tributary: $p < .001$, $t = -22.376$, d.f. = 3
Townsend Basin: $p = .002$, $t = -25.343$, d.f. = 2
Townsend Tributary: $p < .001$, $t = -39.690$, d.f. = 3
High Point City Basin: $p < .001$, $t = -50.473$, d.f. = 3
High Point City Tributary: $p < .001$, $t = -20.408$, d.f. = 3